The response of pyriproxyfen-resistant and susceptible *Bemisia tabaci* Genn (Homoptera: Aleyrodidae) to pyriproxyfen and fenoxycarb alone and in combination with piperonyl butoxide

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Abstract: Pyriproxyfen was effective against susceptible *Bemisia tabaci* eggs at a LC_{50} of 0.003 mg litre^{-1} and against nymphs at 0.02 mg litre^{-1}. In comparison, eggs of a laboratory selected, pyriproxyfen-resistant *B tabaci* strain, originating in an Israeli greenhouse, exhibited 6500-fold resistance and nymphs exhibited 1100-fold resistance. Eggs and nymphs of a strain from an Israeli sunflower field exhibited 450 and 210-fold resistance in comparison to the susceptible standard. Fenoxycarb was generally less effective than pyriproxyfen against *B tabaci* eggs and nymphs but was unaffected by pyriproxyfen resistance. Piperonyl butoxide (PB) was antagonistic to pyriproxyfen, and this increased with increasing pyriproxyfen resistance. PB had no effect on the toxicity of fenoxycarb. Collectively, these data imply that the modes of action of pyriproxyfen and fenoxycarb are distinct, despite the structural similarities of these molecules. Possible reasons for the antagonism of PB against pyriproxyfen are discussed.

Keywords: pyriproxyfen; fenoxycarb; piperonyl butoxide; *Bemisia tabaci;* cross-resistance

1 INTRODUCTION

The juvenile hormone analogues (JHAs) fenoxycarb and pyriproxyfen are similar in structure (Fig 1) and have similar effects on whiteflies. Fenoxycarb is a non-terpenic, non-neurotoxic carbamate with juvenoid activity. It affects egg production and viability, larval development and adult emergence in the homopterans *Trialeurodes vaporariorum* Westwood[1] and *Aleyrodes protella* L[2] and it has been shown to effect good control of *Bemisia tabaci* (Gennadius) in cotton.[3]

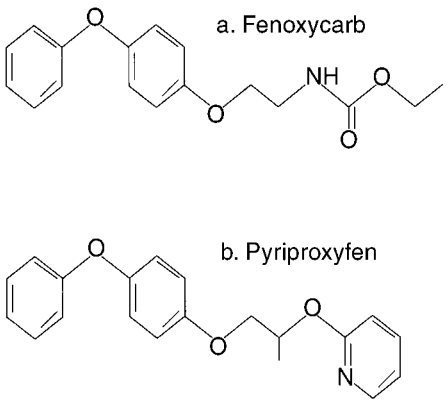

Figure 1. Structures of (a) fenoxycarb and (b) pyriproxyfen.

Pyriproxyfen is a pyridine-substituted form of fenoxycarb with similar but more potent activity than the unsubstituted molecule. This may be because it is less easily hydrolysed and more stable *in vivo*.[4] It affects egg viability and adult emergence in *B tabaci* and *T vaporariorum*[5,6] and is effective in the μg litre^{-1} range, becoming an increasingly common choice for the control of whiteflies resistant to more traditional insecticides.[7,8]

The exact modes of action of fenoxycarb and pyriproxyfen are unknown but, in general, JHAs depress JH biosynthesis in the *corpora allata* and assume the role of endogenous JH.[9] The carbamyl moiety of fenoxycarb is known to inhibit JH-specific

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esterases.[10] Both compounds can suppress the synthesis of lepidopteran proteins associated with female fertility.[11,12] In this respect it is likely that they interfere with the role of JH in gene regulation.[13]

Despite the effectiveness of pyriproxyfen, resistance is now a major concern for the management of *B tabaci* in Israel. Three successive applications in a rose greenhouse in 1991 resulted in over 500-fold resistance at LC_{50} when measured as increased egg viability.[14] Strong resistance is now also being reported from some field populations, on sunflowers and cotton, despite its use being restricted to one application per year (AR Horowitz, unpublished data). This development has major implications for whitefly control in the United States where, in 1996, pyriproxyfen was granted emergency registration for the control of organophosphate- and pyrethroid-resistant *B tabaci*.[8]

To date, no cross-resistance has been found between pyriproxyfen and other novel insecticides such as the chitin synthesis inhibitor, buprofezin and the thiourea derivative, diafenthiuron.[15,16] However, the potential for cross-resistance between pyriproxyfen and fenoxycarb has not been investigated. So far, resistance to fenoxycarb has not been documented, but this may be because it is not used intensively.

In houseflies, oxidases (particularly cytochrome P450s) and reduced penetration have been proposed as resistance mechanisms against pyriproxyfen.[17,18] Piperonyl butoxide (PB), although a well-known inhibitor of microsomal oxidases, is also widely acknowledged to affect insect development,[19–21] and has been shown to affect growth rates in *B tabaci* nymphs.[22,23] Piperonyl butoxide is probably a functional (rather than structural) mimic of JH in that it may increase the titre of this hormone by preventing its oxidative degradation – a mode of action distinct from those proposed for pyriproxyfen or fenoxycarb.

This paper investigates the responses to pyriproxyfen, fenoxycarb and PB of *B tabaci* strains differing markedly in susceptibility to the former, and the consequences of co-applying PB with pyriproxyfen or fenoxycarb against pyriproxyfen-susceptible and -resistant populations.

2 MATERIALS AND METHODS

2.1 *Bemisia tabaci* strains and cotton cultivar

The strains PYRR, PYRS and FR were all of Israeli origin. PYRR was derived from a pyriproxyfen-resistant glasshouse population and had been selected further in the laboratory since 1992 by exposing insects to cotton plants treated with 20 mg litre^{-1} of the chemical on a regular basis. PYRS originated in the field in 1976 and was susceptible to pyriproxyfen. The field-resistant strain (FR) was collected from sunflowers in the Nachshon area of central Israel in May 1996 and tested within four weeks of collection. This area was known to harbour pyriproxyfen-resistant individuals of *B tabaci* (AR Horowitz, unpublished data). All strains were routinely maintained on whole cotton plants at 25($\pm$1)°C, 14 : 10 L : D. The cotton cultivar 'Vered' (Hazerah Ltd, Israel) was used throughout. Plants were infested with whiteflies once they had developed two true leaves (three to four weeks after sowing).

2.2 Bioassay method

Bioassays were conducted on whole cotton plants, at the two-to-three-node stage. Adults were confined to leaves using clip-cages, and allowed to oviposit for 24 h. Areas bounded by the cages were marked with a felt pen, and the cages and adults were removed. Eggs or immature stages were counted prior to treatment so that survival could be measured.

Bemisia tabaci were treated either as zero-to-one-day-old eggs or as 10- to 11-day-old 2nd-instar nymphs. For any bioassay, each treatment was represented by five replicates and each bioassay was repeated twice. Treatments were made by dipping leaves and their attendant eggs or nymphs in serial dilutions of formulated material. Egg-hatch was assessed by comparing the number of eggs present on the day of treatment with the number remaining unhatched 10–12 days later. Second-instar survival was assessed by comparing the number of live pupae and eclosed pupal cases with the number of 10- to 11-day-old nymphs present prior to treatment. All plants and insects were maintained at 25($\pm$1)°C and 65% RH.

2.3 Chemicals

Pyriproxyfen, fenoxycarb and PB were applied as formulated products ('Tiger' 100 g litre^{-1} EC, Sumitomo, 'Insegar' 250 g kg^{-1} WP, Novartis, and PBEC80 800 g litre^{-1} EC, Endura, respectively).

2.4 Statistical analyses

Dose-response data were subjected to probit analysis using the POLO program (LeOra Software, Menlo Park, California). For experiments combining PB with either of the JHAs, these dose-response data identified the optimal JHA concentrations to be used. The maximum concentration of PB utilised in these mixtures was either a moderately toxic one (160 mg litre^{-1}) when treating 2nd instars or a non-toxic one (1000 mg litre^{-1}) when treating eggs. Data derived from these combinative experiments were logit-transformed and analysed using a Genstat® program. This program returned the mean mortalities and associated standard errors. The results were back-transformed and so gave skewed 95% confidence limits.

3 RESULTS

At concentrations of 5000 mg litre^{-1} PB had no lethal effect on the eggs of any of the three strains

Strain	*Stage*	*n*	LC_{50} (*mg litre*$^{-1}$)	*95% CL*	*Slope*
PYRS	Eggs	857	>5000	—	—
	2nd instars	515	185	137–261	1.4
PYRR	Eggs	1196	>5000	—	—
	2nd instars	488	66	45–93	1.0
FR	Eggs	164	>5000	—	—
	2nd instars	683	630	560–705	7.5

Table 1. Effect of PB on zero- to one-day-old eggs and 2nd-instar *Bemisia tabaci* nymphs

tested. This compound was, however, quite toxic to 2nd-instar nymphs. The LC_{50} for the PYRR strain was 66 mg litre^{-1}, significantly lower than those of 185 and 630 mg litre^{-1}, obtained for PYRS and FR strains respectively (Table 1).

When treating zero-to-one-day-old eggs with pyriproxyfen, the resistance factor (RF) exhibited by PYRR was 6500-fold compared with PYRS. For 2nd instars, the corresponding RF of PYRR was greater than 1000 (Table 2). For the PYRR strain, the amount of pyriproxyfen required to kill 2nd-instar whiteflies was similar to that required for PB (LC_{50} values of 34 and 66 mg litre^{-1} respectively). For eggs and nymphs respectively, the FR strain, although not as resistant as PYRR, was still 450- and 210-fold more resistant to pyriproxyfen than PYRS.

Fenoxycarb proved very effective against the eggs of the PYRS strain (LC_{50} = 8 mg litre^{-1}; Table 3), and as effective as PB or pyriproxyfen against PYRR nymphs (LC_{50} = 65 mg litre^{-1}). Generally, fenoxycarb and pyriproxyfen were more effective as ovicides than against nymphs. This contrasted with PB, which caused no mortality of eggs (Table 1). There was evidence for slight tolerance of fenoxycarb by PYRR eggs, as compared with FR or PYRS, but this was not apparent among 2nd instars.

In a comparison of successful egg-hatch (Fig 2a–c) and pupation (Fig 2d–e), there was clear and marked antagonism of pyriproxyfen with the addition of piperonyl butoxide. Antagonism was greater in FR than in PYRS, and greatest of all in PYRR. In the latter strain, concentrations of 1000 mg litre^{-1} of PB caused an 88% increase in egg-hatch in comparison with pyriproxyfen alone (at a fixed concentration of 36.5 mg litre^{-1} pyriproxyfen). This concentration was chosen to achieve ⩾50% mortality in order that the antagonistic effects of PB could be clearly observed. PB also effected increases in successful pupation of 28% and 50% respectively for PYRS and PYRR whitefly. PB had no apparent effect (synergistic or antagonistic) on egg-hatch (Fig 3a–b) or on 2nd-instar survival (Fig 3c–e) in fenoxycarb-treated whitefly.

4 DISCUSSION

Although the lethal effects of PB are seldom acknowledged, this compound reduced survival of *B tabaci*

Strain	*Stage*	*n*	LC_{50} (*mg litre*$^{-1}$)	*95% CL*	*Slope*	*RF*[a]
PYRS	Eggs	486	0.003	0.001–0.004	1.7	—
	2nd instars	635	0.02	0.12–0.03	1.2	—
PYRR	Eggs	491	19.5	12.2–26.4	3.3	6500
	2nd instars	348	34.0	19.5–65.2	2.6	1100
FR	Eggs	833	1.5	0.9–2.1	2.2	450
	2nd instars	677	9.3	5.0–19.7	1.2	210

[a] Resistance factor: LC_{50} PYRR or FR/LC_{50} PYRS

Table 2. Effect of pyriproxyfen on zero- to one-day-old eggs and 2nd-instar *Bemisia tabaci* nymphs

Strain	*Stage*	*n*	LC_{50} (*mg litre*$^{-1}$)	*95% CL*	*Slope*	*TF*[a]
PYRS	Eggs	514	7.7	5.4–10.6	1.7	—
	2nd instars	745	115	57.9–184	2.0	—
PYRR	Eggs	729	34.0	18.6–64.5	1.3	4
	2nd instars	382	65.4	37.5–140.3	1.3	0.6
FR	Eggs	2715	4.7	3.0–6.6	1.8	0.6
	2nd instars	473	73.3	28.5–125.4	2.2	0.6

[a] Tolerance factor: LC_{50} PYRR or FR/LC_{50} PYRS

Table 3. Effect of fenoxycarb on zero- to one-day-old eggs and 2nd-instar *Bemisia tabaci* nymphs

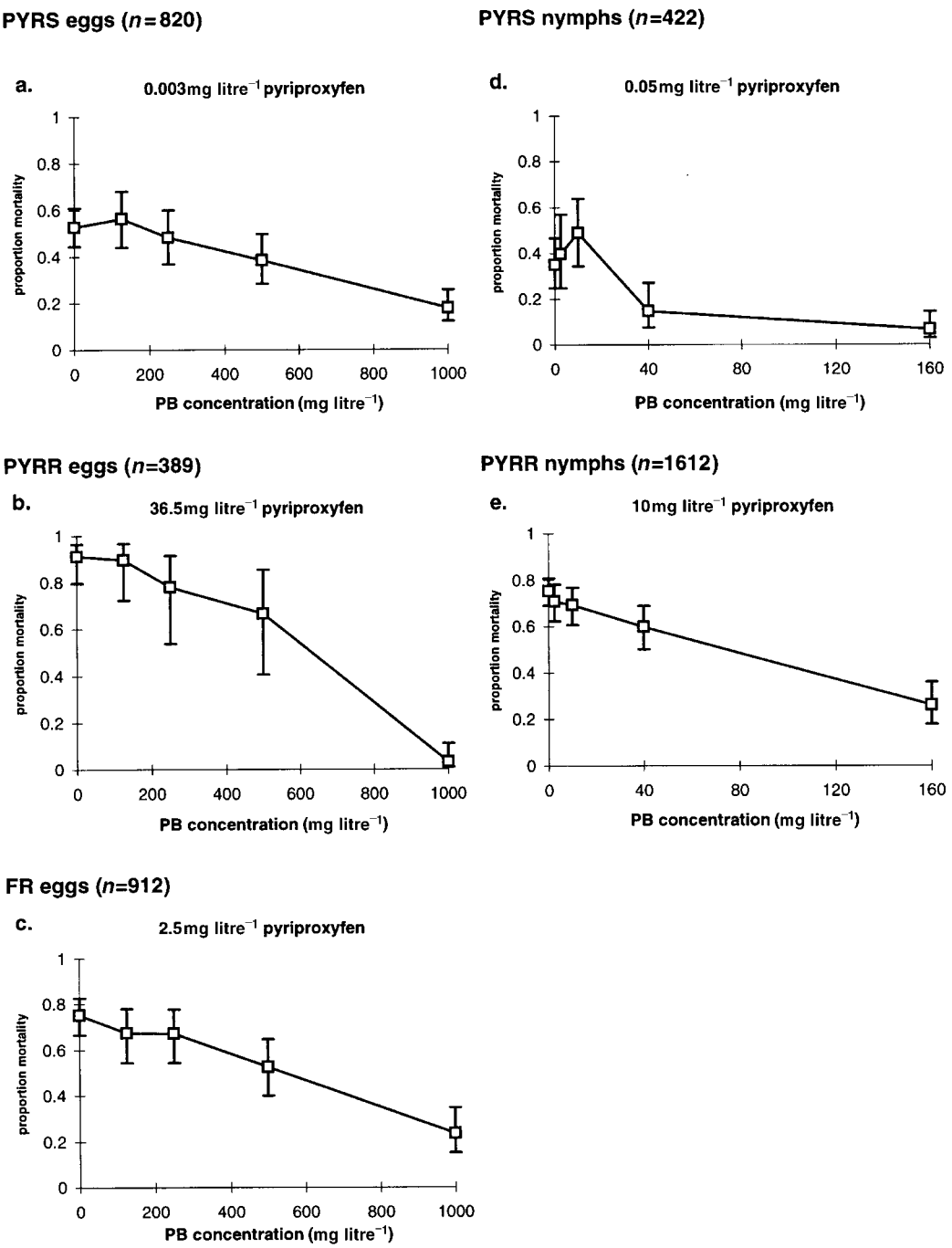

Figure 2. The effect of concentration of PB on the toxicity of a fixed concentration of pyriproxifen to nymphs and eggs of three *Bemisia tabaci* strains (mean mortality and 95% confidence limits).

nymphs to the extent predicted from results of Devine and Denholm[23] who found LC_{50} values for a number of strains to range between *c* 60 and 600 mg litre^{-1} for second instar nymphs.

Results for pyriproxyfen toxicity bear comparison with those obtained by Ishaaya and Horowitz[5] who examined the transovarial effects of pyriproxyfen on egg-hatch (by exposing adults to residues for 48 h) and reported an LC_{50} of 0.026 mg litre^{-1} pyriproxyfen for a susceptible strain. This is 9-fold higher than that obtained for egg-hatch in this study (0.003 mg litre^{-1}). The discrepancy is possibly due to the more direct method of application (to eggs rather than adults) adopted here. Results for 2nd instars were more comparable. Horowitz and Ishaaya[14] found that 0.01 mg litre^{-1} pyriproxyfen prevented 50% of nymphs from emerging as adults. This is comparable to a corresponding LC_{50} value of 0.02 mg litre^{-1} gained in the present study. The PYRR strain used here was descended from one tested in 1993/1994 by Ishaaya and Horowitz,[16] who found a resistance factor of 757 based on transovarial effects on egg-hatch. In the present work, resistance to pyriproxyfen in the PYRR strain was appreciably higher, most likely a consequence of continued laboratory selection of this strain by pyriproxyfen.

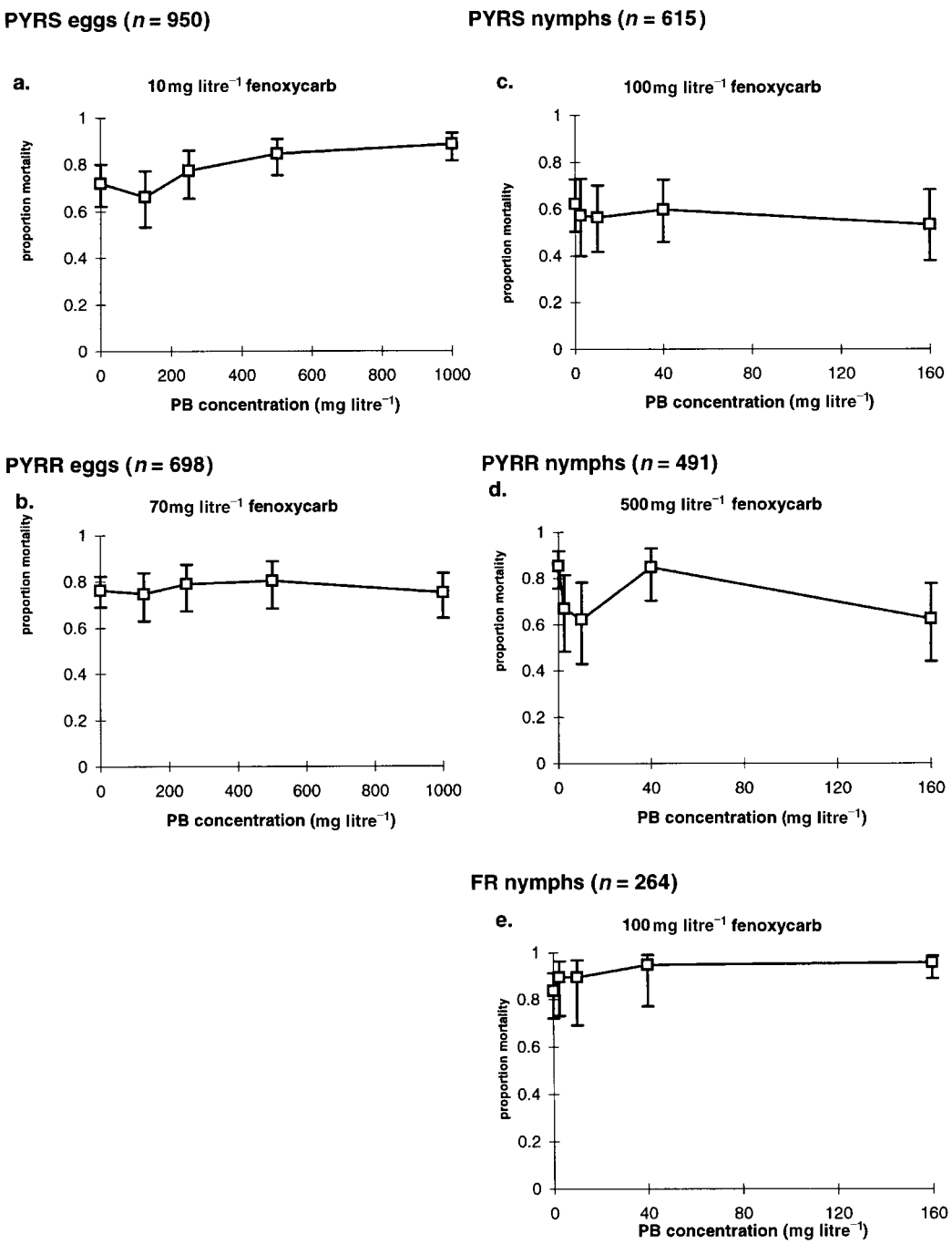

Figure 3. The effect concentration of PB on the toxicity of a fixed concentration of fenoxycarb to nymphs and eggs of three *Bemisia tabaci* strains (mean mortality and 95% confidence limits).

The field strain, FR, also exhibited resistance to pyriproxyfen, despite ongoing and severe restrictions on the use of this chemical on field crops.[7] Reasons for the selection of resistance in this population are unclear but of great concern; further work is under way to determine the incidence of resistance at several field sites in Israel and to investigate factors (eg ecological isolation, proximity to greenhouses etc), that may account for its occurrence and characterisation.

PB had no effect on mortality caused by fenoxycarb, but was very antagonistic to pyriproxyfen. This implies that the modes of action of fenoxycarb and pyriproxyfen are quite distinct and that PB-suppressible microsomal oxidases are not involved in the breakdown of pyriproxyfen to less toxic metabolites. The antagonism is difficult to explain but it is possible that oxidation is required to produce a pyriproxyfen metabolite more toxic than the parent compound, and that this process is inhibited by PB. Solomon and Metcalf[24] reported that PB can inhibit oxidation of another JHA, methoprene, to a more active metabolite in the homopteran *Oncopeltus fasciatus* (Dallas). If this hypothesis is correct, whitefly strains with lower titres of oxidases, or treated with an oxidase inhibitor such as PB, would be less sus-

ceptible to pyriproxyfen. Of the three strains, PYRR was most resistant to pyriproxyfen and most susceptible to PB. This greater susceptibility to PB could imply that pyriproxyfen resistance is conferred, in part at least, by the presence of low oxidase titres preventing its conversion to a more toxic metabolite. However, Zhang *et al*[17] reported that resistance to pyriproxyfen in houseflies was correlated with the presence of genes coding for the production of P-450 enzymes. In that situation, one would expect PB to synergise pyriproxyfen, but they found no effect. However, another inhibitor, propynyl trichlorophenyl ether, did synergise pyriproxyfen.

Unexpectedly for two such closely related compounds, there was no clear cross-resistance between fenoxycarb and pyriproxyfen in either FR or PYRR. In a comparison of ovicidal effects, PYRR exhibited only a four-fold tolerance to fenoxycarb over PYRS and nymphicidal effects occurred at similar concentrations for all three strains. In principle, therefore, there are opportunities in Israel for substituting or alternating pyriproxyfen with fenoxycarb, applied alone or in combination with an adulticide. The dissimilarity in the patterns of response by whitefly strains to fenoxycarb, pyriproxyfen and PB (ie no correlation in tolerance between the strains), suggests that resistance to pyriproxyfen does not affect the response of *B tabaci* to either fenoxycarb or PB. However, it should be stressed that this conclusion applies only to the mechanism of pyriproxyfen resistance currently present in *B tabaci* in Israel. Other mechanisms of resistance may arise, and it is conceivable that these, or ones selected specifically by fenoxycarb, will confer more significant cross-resistance between the two chemicals. At present it is impossible to assess the wider applicability of our findings, since we are unaware of any reports of significant resistance by *B tabaci* to fenoxycarb, or of proven cases of pyriproxyfen resistance from elsewhere in the world.

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